Patch clamp study of the UNC-105 degenerin and its interaction with the LET-2 collagen in *Caenorhabditis elegans* muscle

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Degenerins have emerged from genetic studies in *Caenorhabditis elegans* as candidate mechanically gated amiloride-sensitive ion channels for transducing mechanical stimuli into cellular responses. In *C. elegans* muscle, the existence of a genetic interaction between the *unc-105* degenerin gene and *let-2*, a gene encoding an α2(IV) collagen, raised the possibility that UNC-105 may function as a mechanically gated channel in a stretch receptor complex. However, to date, ion channel activity of UNC-105 has only been recorded in a gain-of-function mutant form in heterologous expression systems. In this study we investigated the *in situ* properties of UNC-105 using the whole cell configuration of the patch clamp technique on body wall muscle cells from acutely dissected *C. elegans*. Amiloride was found to be without effect on membrane potential of wild-type muscle cells, suggesting that the UNC-105 degenerin is electrically silent in resting muscle. Hypo-osmotic shocks induced a reversible depolarization of muscle cells but which was not affected by amiloride. Deformation of the cells by applying tension to the filamentous complex on which muscle cells remained attached or by ejecting external solution under pressure failed to induce any change of membrane potential. In gain-of-function *unc-105(n506)* mutant cells, an amiloride-sensitive inward Na^+ current was found to be constitutively active, leading to maintained muscle depolarization. An associated mutation in the α2(IV) collagen LET-2 led to the closure of the mutant UNC-105(n506) channel while a collagenase treatment of these double mutant cells caused it to re-open, giving evidence for a functional interaction between LET-2 collagen and mutant UNC-105 channel.

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Little is known about the molecules involved in mechanotransduction, the process that converts mechanical stimuli into cellular responses. Genetic analysis of the *Caenorhabditis elegans* model system has nevertheless proved fruitful in identifying genes implicated in this process. Extensive genetic screens of *C. elegans* have indeed revealed about 15 genes which, when mutated, disrupt gentle body touch sensation (Chalfie & Sulston, 1981; Chalfie & Au, 1989). A number of these genes, *mec-4*, *mec-10*, *unc-8*, *unc-105, del-1* and *deg-1*, encode proteins that share a common topology with amiloride-sensitive epithelial Na^+ channels consisting of two membrane-spanning domains separated by a long extracellular loop (Chalfie & Wolinsky, 1990; Driscoll & Chalfie, 1991; Canessa *et al.* 1993; Huang & Chalfie, 1994; Liu *et al.* 1996; Tavernarakis *et al.* 1997). These channels have been proposed to be mechanically gated and to constitute the core of a mechanotransducing complex (Waldmann & Lazdunski, 1998; Tavernarakis & Driscoll, 2001; Kellenberger & Schild, 2002; Goodman & Schwarz, 2003; Strange, 2003). Except for *unc-105* expressed in body wall muscle, specific gain-of-function mutations in the genes encoding these proteins induce swelling and subsequent degeneration of the neurones in which they are expressed. This led to the designation of this *C. elegans* gene family as the degenerin family (Driscoll & Chalfie, 1991). Gain-of-function mutations in the degenerin gene *unc-105* do not cause muscle degeneration but rather induce hypercontraction (Park & Horvitz, 1986*a*). To date, two *C. elegans* degenerins, UNC-105 and coexpressed MEC-4/MEC-10, have been demonstrated to function as amiloride-sensitive Na^+

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channels in heterologous expression systems (Garcia-Anoveros *et al.* 1998; Goodman *et al.* 2002). However, only mutant variants of the channels containing the 'degenerin' mutation were found to be constitutively active or, in the case of MEC-4/MEC-10, the stomatin-related protein MEC-2 needed for touch sensitivity allowed current to be detected with wild-type MEC-4/MEC-10. Moreover, attempts to gate these channels in expression systems were unsuccessful. Yet, many genetic observations serve working models in which the degenerin channels may be mechanically gated. According to such models, MEC-4/MEC-10 may constitute the core of a mechanosensory complex in sensory neurones, tethering extracellular matrix and intracellular cytoskeleton to the degenerin channel and thus conferring gating tension on the channel (Hong & Driscoll, 1994; Huang & Chalfie, 1994; Gu *et al.* 1996). In addition, using the genetically encoded Ca^{2+} indicator cameleon to monitor the activity of touch neurones, it was recently demonstrated that the channel subunit MEC-4 and the associated stomatin MEC-2 were required for generating Ca^{2+} transients in response to gentle touch (Suzuki *et al.* 2003). In body wall muscle, the *unc-105* gene was shown to interact with *let-2* (Liu *et al.* 1996), which encodes an α2-like chain of collagen IV belonging to the muscle basement membrane (Sibley *et al.* 1993; Graham *et al.* 1997). On the basis of this genetic interaction, it was postulated that UNC-105 may also be a mechanically gated channel, LET-2 carrying tension to UNC-105 (Liu *et al.* 1996). However, electrophysiological approaches failed to demonstrate mechanosensory channel activity in heterologous expression systems, most likely because the functional reconstitution of the whole complex required for mechanosensory transduction is impossible in expression systems. The measurement of degenerin channel activity in its native environment has thus become a major challenge. *In situ* physiological studies are nevertheless greatly restricted in *C. elegans* because of the difficulty of exposing touch neurones or body wall muscle cells where degenerins are present.

In this paper, using the whole cell configuration of the patch clamp technique on acutely dissected worms, we investigated the gating properties of UNC-105 channels *in situ* in body wall muscle cells from *C. elegans*. We demonstrated that wild-type UNC-105 channels remain closed in resting muscle and are insensitive to mechanical stimuli under our experimental conditions. A gain-of-function mutation gave rise to the constitutive activation of an amiloride-sensitive Na^+ current causing a maintained depolarization of muscle cells. We also provide experimental evidence that the α2(IV) collagen LET-2 functionally interacts with UNC-105 since an associated mutation in LET-2 repressed the constitutive activity of UNC-105 while a collagenase treatment led to re-opening of the mutant channel.

Methods

Strains

Experiments were performed on the N2 wild-type reference and the *unc-105(n506)* strains. *unc-105(n506); let-2(n821)* (or initially *unc-105(n506); sup-20(n821)*; Park & Horvitz, 1986*b*) was constructed by crossing MT1720 *unc-105(n490); let-2(n821)* with *unc-105(n506); lin-15(n765)* and keeping animals not carrying *lin-15* in their progeny. DNA from *unc-105(n506); let-2(n821)* homozygous animals was amplified by PCR using standard protocols and sequenced on a Megabace analyser (Amersham). In the course of these experiments, it was noticed that the *n506* mutation is a Glu to Lys mutation affecting amino acid 673 and not amino acid 677 (amino acids 635 and 639, respectively, in Wormbase/GenBank sequences) as mentioned in Liu *et al.* (1996).

Electrophysiology

The dissection technique was performed as previously described (Jospin *et al.* 2002*a*,*b*). Briefly, adult nematodes were glued by applying a cyanoacrylic glue along one side of the body. An incision was made in the cuticle using a sharpened tungsten rod. The viscera were cleared and the cuticle flap was pushed down with a glass rod held by a micromanipulator (see Fig. 2*B* and online Supplementary material movie no. 1). Membrane currents and potentials were recorded in the whole cell configuration on ventral body wall muscle cells using a patch clamp amplifier (model RK 400; Bio-Logic, Claix, France). The resistance of recording pipettes was within 2–3 MΩ. Acquisition and generation of command voltage pulses were done using Biopatch software (Bio-Logic) driving an A/D, D/A converter (LabMaster DMA board, Scientific Solutions Inc., Mentor, OH, USA). Currents and potential differences were analysed using Origin software (OriginLab Corp., Northampton, MA, USA). Linear resistive and capacitive components were not compensated. Cell capacitance was determined by integration of a control current trace obtained with a 10 mV depolarizing pulse from −70 mV. This capacitance was used to calculate the density of Na^+ currents (A F^{-1}) and the input resistance normalized to the capacitance (MΩ pF^{-1}). In a preceding study, the voltage was calculated to be uniform and the cell isopotential

under these experimental conditions (Jospin *et al.* 2002*a*). All experiments were carried out at room temperature (19–23°C).

Imaging

Cells were imaged using a ×20 objective on an inverted microscope (Olympus IMT2). Images from a region of interest (480 × 480 pixels) were captured with a Coolsnap$_{fx}$ charge-coupled device camera (Roper Scientific, Tucson, AZ, USA) at a frequency of 6 Hz. Image acquisition and processing were performed using the MetaVue imaging workbench (Universal Imaging Corp., Downingtown, PA, USA).

Solutions and chemicals

Pipettes were filled with (mM): 120 KCl, 20 KOH, 4 $MgCl_2$, 5 TES, 4 Na_2ATP, 36 sucrose, 5 EGTA, pH 7.2. The bath solution corresponded to a modified Tyrode solution containing (mM): 140 NaCl, 5 KCl, 6 $CaCl_2$, 5 $MgCl_2$, 11 glucose and 5 Hepes, pH 7.2. $CaCl_2$ and $MgCl_2$ were omitted in the divalent cation-free Tyrode solution. For hypo-osmotic experiments, the hypo-osmotic shock was applied by replacing a Tyrode solution containing 60 mM NaCl and 160 mM sucrose instead of 140 mM NaCl by a hypo-osmotic solution containing 60 mM NaCl and no sucrose. Amiloride (Sigma) and type I collagenase (6 mg ml^{-1}) (Sigma) were diluted to the required concentrations in the bath solution. Voltages were not corrected for liquid junction potentials calculated to be lower than 5 mV with the different solutions used.

Statistics

Data values are presented as means ± S.E.M. Data were statistically analysed using Mann-Whitney test except when mentioned. Values were considered significant when $P < 0.05$. On figures, one asterisk (*) indicates $P < 0.05$, three asterisks (***) indicate $P < 0.0005$ and n.s. a non-significant difference.

Results

Figure 1*A* (left panel) shows a continuous recording of the internal potential of a body wall muscle cell from a wild-type worm under current clamp conditions using the whole cell configuration of the patch clamp technique. The input cell resistance was also monitored by injecting 1 s-duration hyperpolarizing current pulses every 5 s. The internal potential was stabilized around −20 mV and the input resistance was 0.7 GΩ. In this cell, as well as in the seven other cells tested under these experimental conditions, addition of 1 mM amiloride to the extracellular medium did not induce any significant change in the

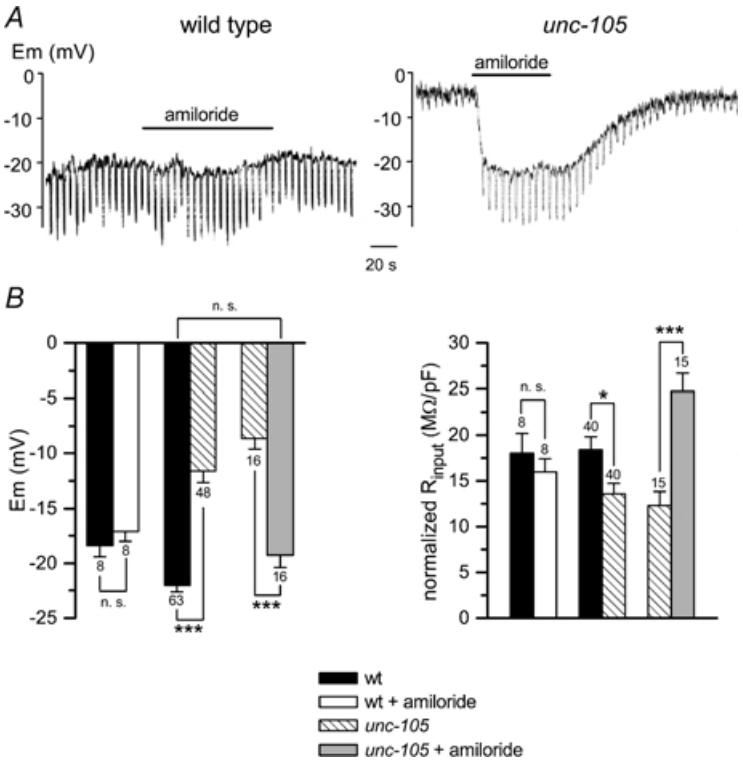

Figure 1. Effects of amiloride on membrane potential and input resistance of wild-type and *unc-105(n506)* cells
A, chart recording of membrane potential in cells stimulated in the current clamp mode by 1 s-duration negative current steps of 20 and 10 pA in a wild-type and in an *unc-105(n506)* cell, respectively. *B*, comparison of mean membrane potential (left panel) and of mean normalized input resistance (right panel) in wild-type and *unc-105(n506)* cells in the absence and in the presence of amiloride. *$P < 0.05$; ***$P < 0.0005$; n.s., non-significant difference.

internal potential and in the input resistance of the muscle cell (Fig. 1*B*). In five cells tested, addition of amiloride had also no significant effect on the whole cell background current recorded at membrane potential ranging from −90 to −30 mV under voltage clamp conditions (not shown). These results suggest that amiloride-sensitive ion channels are not active in resting wild-type muscle cells.

unc-105 has been shown to interact with *let-2*, a gene which encodes a muscle basement membrane α2(IV) collagen (Sibley *et al.* 1993; Liu *et al.* 1996; Graham *et al.* 1997). On the basis of this genetic interaction and of the homology of UNC-105 to other strong candidate mechanosensitive channels, UNC-105 has been proposed to be mechanically gated, LET-2 carrying tension to UNC-105. We therefore explored this possibility in wild-type cells using the current clamp configuration, where, given the high cell input resistance, even a minute Na^+ current would induce a detectable depolarization. The effects of swelling muscle cells were first tested by superfusing cells with solutions having reduced osmolarity. Figure 2*A* shows that reducing osmolarity from 340 to 180 mosmol l^{-1} gave rise to a reversible depolarization from −27 to −22 mV but which was not affected by amiloride. In the 16 cells tested under these conditions, the mean hypo-osmotic-induced depolarization was 5.8 ± 1.4 mV and in every case amiloride did not alter these depolarizations. On the basis of genetic data, we suspected that mechanical gating of the channel required transduction of the tension change through interactions with components in the basement membrane underlying muscle cells. We first tried to shift the rod used in the dissecting operation to flatten the cuticle (see Methods) to apply tension to the whole

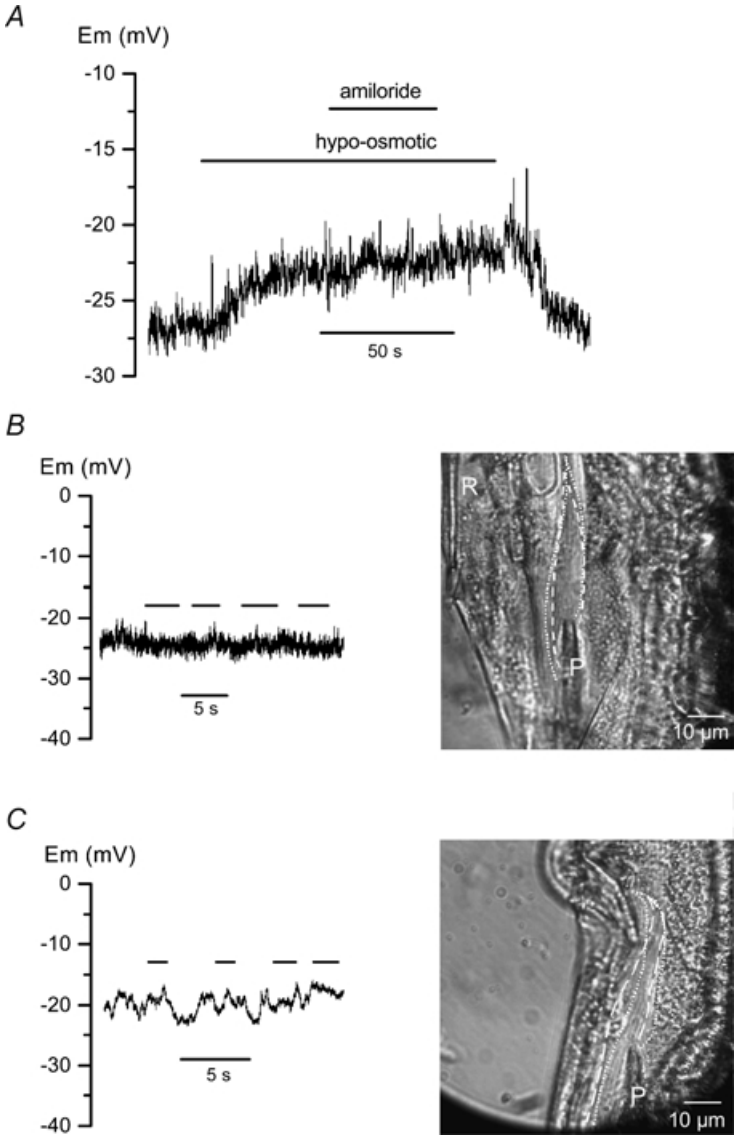

Figure 2. Effects of various mechanical stimuli on membrane potential of wild-type cells
A, the lower bar above the voltage trace indicates the period during which a hypo-osmotic (180 mosmol l^{-1}) solution was superfused. *B*, bars above the voltage trace indicate the period during which the glass rod used to maintain the cuticle open was shifted to apply tension and deform the muscle cell. The corresponding light micrograph shows the muscle cell (outlined by dashes) with the recording pipette (P) before the glass rod (R) was shifted. The new position of the muscle cell after the glass rod had been moved (upwards in the field) is indicated by the dotted line (see also online Supplementary material, movie no. 1). *C*, bars above the voltage trace indicate the period during which external solution was ejected under pressure through a glass capillary. In the corresponding light micrograph, the position of the patched muscle cell is outlined by dashes and dots before and during ejection of external solution, respectively (see also online Supplementary material, movie no. 2). The glass capillary was placed on the left but is not visible at this magnification.

filamentous complex on which muscle cells remained attached. The extent of deformation was simultaneously monitored from digital images taken with a camera. As illustrated in Fig. 2*B*, shifting the glass rod up to compromise the pipette seal did not affect the membrane potential (mean membrane potential of -22.7 ± 1.2 mV; $n = 12$) while the muscle cell was extensively deformed (see also Supplementary material, movie no. 1). Finally, using a glass capillary whose mouth was placed in the close vicinity of the patched cell (less than 50 μm), external solution was ejected under pressure to deform the cell. Although this operation induced a detectable bending of the cell as shown in Fig. 2*C* (see also Supplementary material, movie no. 2), it had no effect on the membrane potential (mean membrane potential of -22.2 ± 2.7 mV; $n = 5$).

Gain-of-function mutations in the *unc-105* gene have been shown to cause muscle hypercontraction and paralysis (Park & Horvitz, 1986*a*). Among the different mutant alleles of the *unc-105* gene, we selected one gain-of-function allele, *n506*, which causes body wall muscle hypercontraction and paralysis less severe than those observed for other *unc-105* mutants. A striking observation was that the mean resting potential was significantly less negative in *unc-105(n506)* (-11.6 ± 1.1 mV; $n = 48$) than in wild-type muscle cells (-22 ± 0.6 mV; $n = 63$) (Fig. 1*B*). This less negative membrane potential in *unc-105(n506)* cells probably resulted from the persistent activation of an amiloride-sensitive inward current, since, under current clamp conditions, addition of amiloride induced a reversible hyperpolarization. In the mutant cell presented in the right panel of Fig. 1*A*, amiloride produced a hyperpolarization from -3 to -22 mV associated with an increase in the membrane input resistance from 0.3 to 1.1 GΩ. On average, the mean resting potentials of *unc-105(n506)* cells in the presence of amiloride were not significantly different from those recorded in wild-type cells in its absence indicating that amiloride has brought the internal potential to wild-type values in closing the amiloride-sensitive current (Fig. 1*B*).

To further characterize the amiloride-sensitive current in *unc-105(n506)* cells, the effects of amiloride were tested under voltage clamp conditions on currents elicited by voltage steps ranging from -90 to -30 mV in 10 mV increments (Fig. 3*A*). Higher membrane potentials were not tested to prevent Ca^{2+} and K^+ voltage-dependent conductances from being activated (Jospin *et al.* 2002*a*,*b*). The mean current–voltage relationship obtained in control solution displayed an outward rectification (Fig. 3*B*, filled symbols). Amiloride blocked the inward background current such that the mean current–voltage relationship

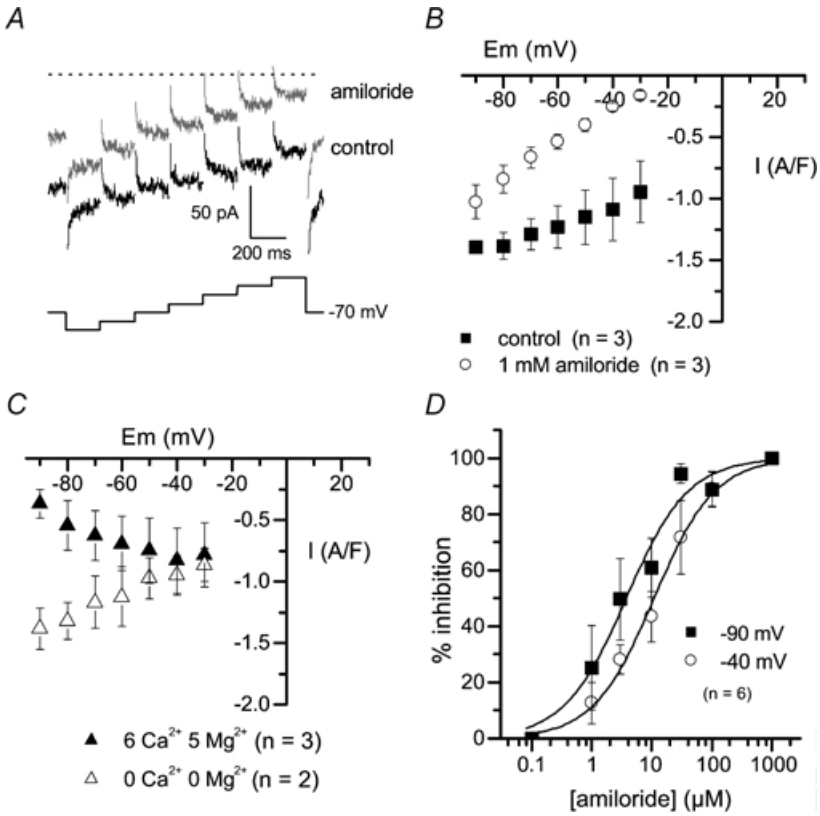

Figure 3. Biophysical and pharmacological properties of the amiloride-sensitive current in *unc-105(n506)* cells
A, membrane currents elicited by 20-ms voltage steps delivered from -90 to -30 mV in 10- mV increments from -70 mV under voltage clamp conditions in control (black trace) and in the presence of amiloride (1 mM) (grey trace). The dotted line indicates the zero current level. *B*, corresponding mean current–voltage relationships. *C*, mean current–voltage relationships of the amiloride-sensitive current in the presence and absence of external divalent cations. *D*, dose–response curves of amiloride block in *unc-105* cells at two different voltages. The best fits to the mean data were adjusted using a Hill equation, with a Hill coefficient of 0.84 and 0.86 and a $K_{0.5}$ of 4 and 11 μM at -90 and -40 mV, respectively.

of the amiloride-sensitive component exhibited a negative slope at negative membrane potentials (Fig. 3*C*, filled symbols). The blocking action of amiloride is known to be voltage dependent but stronger at negative membrane voltages which thus cannot explain the observed negative slope. However, a similar negative slope was observed with gain-of-function mutant UNC-105(A692V) amiloride-sensitive Na^+ channels expressed in *Xenopus* oocytes or HEK-293 cells which resulted from a voltage-dependent block by external Ca^{2+} and Mg^{2+} (Garcia-Anoveros *et al.* 1998). The blockade increased with hyperpolarization so that, in the absence of external Ca^{2+} and Mg^{2+}, the current–voltage relationship became approximately linear. We therefore analysed the voltage dependence of the amiloride-sensitive component in the absence of external divalent cations. Under these conditions, the current–voltage relationship of the amiloride-sensitive component in *unc-105(n506)* cells approached linearity as observed with expressed channels (Fig. 3*C*, open symbols). These results also stressed the Na^+ selectivity of the amiloride-sensitive current. Indeed, assuming linearity over the whole voltage range, the mean reversal potential was extrapolated to $+64 \pm 13.1$ mV ($n = 2$) (Fig. 3*C*), a value close to the calculated equilibrium potential for Na^+ ($+73$ mV) which, in the absence of external Ca^{2+}, was the only ion whose equilibrium potential had positive value.

Amiloride was found to block inward background currents in *unc-105(n506)* cells with an apparent half-blocking concentration of 4 μM at -90 mV (Fig. 3*D*). The block was voltage dependent since the half-blocking concentration of amiloride was shifted toward a significantly higher value at -40 mV (Wilcoxon test, $P = 0.043$). Finally, as observed for wild-type cells, we failed to modulate mutant UNC-105(n506) channel opening by mechanical stimuli applied either directly to the cell or indirectly to the cuticle.

The hypercontraction phenotype caused by gain-of-function mutations in *unc-105* has been shown to be suppressed by a point mutation in *let-2* (Park & Horvitz, 1986*b*; Liu *et al.* 1996). In order to clarify the nature of the interaction that is supposed to exist between UNC-105 and LET-2, we examined the electrical properties of

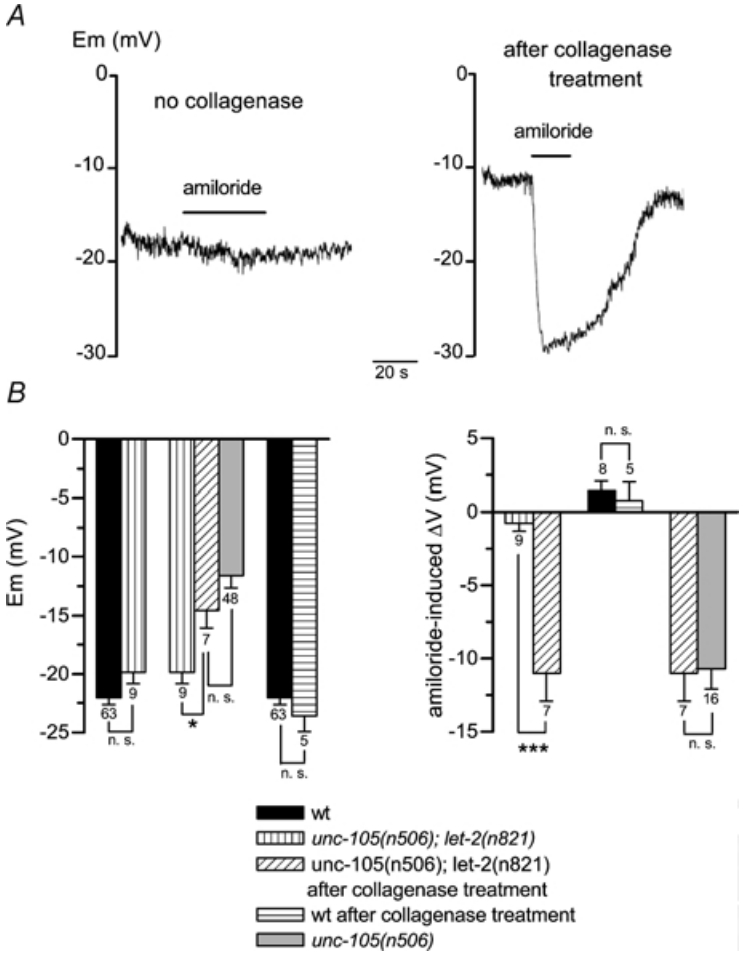

Figure 4. Effect of amiloride and collagenase on membrane potential of double mutant *unc-105(n506); let-2(n821)* cells
A, chart recording of membrane potential in a double mutant cell non-treated with collagenase (left panel) and in another double mutant cell after treatment with collagenase (right panel). *B*, comparison of the mean membrane potential (left panel) and of the mean amiloride-induced voltage change (right panel) in wild-type and *unc-105(n506); let-2(n821)* cells before and after collagenase treatment.

the double mutant carrying the *unc-105* mutation plus the *let-2* suppressing mutation. As reported by Park & Horvitz (1986*b*), the phenotype of the *unc-105(n506); let-2(n821)* strain was found to be wild-type. The mean membrane potential of these double mutant cells (-19.9 ± 0.9 mV; $n = 9$) was not significantly different from the value measured in wild-type worms (Fig. 4*B*). Also, as expected, amiloride had little effect on the membrane potential and the amiloride-induced change in voltage was not different from that obtained in wild-type cells (Fig. 4*A* and *B*). A likely explanation is that the *let-2* mutation induces the closure of UNC-105(n506) channels. To test this hypothesis, we tried to disconnect the collagen from the channel by superfusing the cell with collagenase. A 5-min treatment caused the double mutant cells to detach from the cuticle suggesting that the collagenic connection between muscle cell and hypodermis was lost. Interestingly, the mean resting potential of these detached double mutant cells was significantly more depolarized after collagenase treatment (-14.6 ± 1.5 mV; $n = 7$) than before (-19.9 ± 0.9 mV; $n = 9$) and overall was found to be not significantly different from the one of *unc105(n506)* cells (-11.6 ± 1.1 mV; $n = 48$) (Fig. 4*B*). More importantly, detached cells were found to be highly sensitive to amiloride. Figure 4*A* shows that amiloride did not affect the membrane potential, here around -20 mV, of an *unc-105(n506); let-2(n821)* cell not treated with collagenase while amiloride produced a hyperpolarization from -10 to -28 mV after a collagenase exposition. On average, amiloride produced a hyperpolarization of significantly higher magnitude (-11 ± 1.9 mV; $n = 7$) after collagenase treatment than before (-0.8 ± 0.5 mV; $n = 9$) (Fig. 4*B*). The mean hyperpolarization induced by amiloride in double mutant cells after collagenase treatment was not significantly different from the one recorded in *unc-105(n506)* cells suggesting that the degradation of collagen had re-opened the UNC-105 mutated channel (Fig. 4*B*). In wild-type cells, although collagenase did induce cell detachment, the mean resting potential was not significantly changed and cells remained insensitive to amiloride, ruling out a possible-non-specific effect of collagenase on electrical properties (Fig. 4*B*).

Discussion

This study gives the first detailed description of the properties of a degenerin in its physiological environment. The UNC-105 degenerin has been proposed to function as an ion channel in body wall muscle of *C. elegans* because of its high sequence similarity with amiloride-sensitive ion channels (Liu *et al.* 1996). We demonstrate here that the wild-type UNC-105 protein is electrically silent *in situ* in resting muscle since amiloride at a high concentration, which blocks related channels in the same superfamily, had no effect on electrical properties of body wall muscle cells. This result relates to the absence of detectable current resulting from expression of the wild-type UNC-105 protein in *Xenopus* oocytes and human embryonic kidney cells (Garcia-Anoveros *et al.* 1998). Moreover, under our experimental conditions, body wall muscle cells remain firmly attached to the cuticle *via* the hypodermis (see Supplementary material, movie no. 1) so that the absence of current cannot be ascribed to a lack of mechanical connection, as postulated for expression systems. Besides, although we cannot totally exclude that our assays did not reproduce the physiological conditions required for properly activating the channel or that some key component could be disrupted during the dissection procedure, our results suggest that UNC-105 is not involved in a muscle stretch receptor complex (Liu *et al.* 1996). Indeed, deforming the cell directly or indirectly by manipulating the filamentous complex attached to muscle cells did not induce any detectable change in membrane potential or induced changes insensitive to amiloride. Also in connection with these data, Park & Horvitz (1986*a*) described that the null phenotype of *unc-105* was wild-type indicating that UNC-105 does not contribute significantly to muscle function. Our data together with

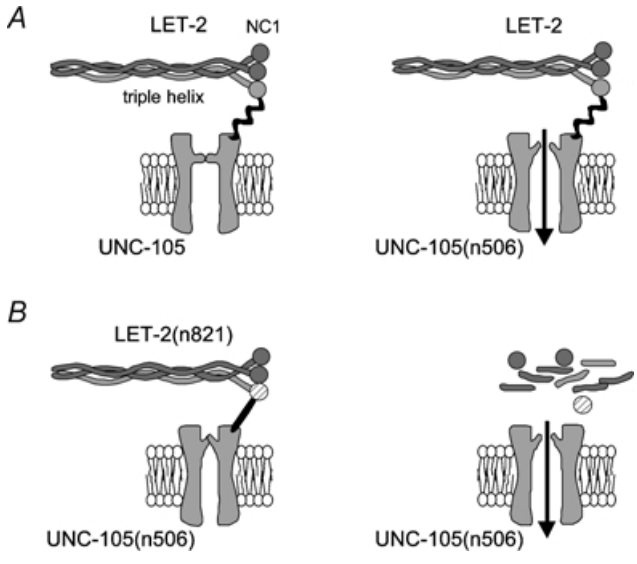

Figure 5. Model of LET-2/UNC–105 interaction
A, in the presence of wild-type LET-2 (in light grey), the α2 chain of the trimeric collagen IV (which may also contain two α1 chains (dark grey); Graham *et al.* 1997), wild-type UNC-105 is closed while the *n506* gain-of-function mutation in UNC-105 constitutively activates the channel. *B*, the *let-2(n821)* mutation in the NC1 domain (hatched) leads to mutant channel closure and degradation of LET-2(n821) by collagenase causes mutated UNC-105(n506) to re-open.

these observations might suggest that UNC-105 remains closed in resting as well as in contracting muscle of *C. elegans*.

We showed that the UNC-105 protein operated as an ion channel in its native cellular environment when mutated. The *unc-105(n506)* mutation here explored consists of the substitution of glutamic acid for lysine at residue 635 near the second membrane-spanning domain (see Methods), close to a conserved region important for gating. Such a mutation resulted in the persistent activation of an amiloride-sensitive Na^+ current that brought the membrane potential to stabilized depolarized values. In the presence of external divalent cations, the current–voltage relationship of the amiloride-sensitive component of the background current displayed a negative slope at negative potentials. It became linear and reversed close to the Na^+ equilibrium potential after removal of external divalent cations. Constitutive activity of gain-of-function mutated UNC-105 channels like UNC-105(A692V) and UNC-105(P134S) was also reported in heterologous expression systems (Garcia-Anoveros *et al.* 1998). Moreover the biophysical properties of the *unc-105(n506)* mutant recorded here in its physiological environment closely matched those reported for the gain-of-function mutated UNC-105(A692V) channel in expression systems (Garcia-Anoveros *et al.* 1998). We found an affinity for amiloride for the *unc-105(n506)* channel of 4 μM at −90 mV, in a range close to the one reported for other amiloride-sensitive channels at the same voltage (see for review Kellenberger & Schild, 2002). The affinity for the drug decreased with depolarization as classically observed for other channels in the same superfamily (see, e.g. Garty & Palmer, 1997). The maintained depolarized value of the mean resting potential of *unc-105(n506)* cells, around −10 mV, may well account for the hypercontracted phenotype of the mutant worms. A membrane potential of −10 mV is indeed much above the threshold for activation of voltage-dependent Ca^{2+} channels (around −20 mV) which control body wall muscle function in *C. elegans* (Jospin *et al.* 2002*a*). This maintained depolarization may induce a constant influx of Ca^{2+} responsible for a tonic contraction of muscle cells which may impair proper locomotion of the worm.

As observed for wild-type cells, we failed to modulate mutant UNC-105(n506) channel opening by mechanical stimuli applied either directly to the cell or indirectly to the cuticle. Yet, *unc-105 has* been reported to interact with *let-2*, a gene encoding an α2(IV) collagen present in the muscle basement membrane (Sibley *et al.* 1993; Liu *et al.* 1996; Graham *et al.* 1997). Indeed, the hypercontraction phenotype caused by gain-of-function mutations in *unc-105* could be suppressed by a mutation in *let-2* (Park & Horvitz, 1986*b*), consisting of the replacement of a highly conserved arginine by a lysine in the NC1 domain of this collagen (Liu *et al.* 1996). In agreement with these genetic data, we demonstrated experimentally that mutant LET-2(n821) did interact functionally with mutant UNC-105(n506) channel since the *let-2(n821)* mutation led to the closure of UNC-105(n506) channels. Moreover, although we cannot exclude that collagenase may have had additional effects on the channel itself, we showed that the degradation of collagen IV by a treatment with collagenase caused the membrane potential to return to a value close to the one recorded in *unc-105(n506)* cells suggesting that the channel had recovered its constitutive activity produced by the gain-of-function mutation. These findings thus do not favour the hypothesis that the mutant UNC-105 channel exaggeratedly opens in response to stretch-induced distortion transduced by LET-2 as postulated by Liu *et al.* (1996), because the likely loss of the connection between LET-2 and UNC-105 after collagenase treatment did not induce closure but in contrast caused the channel to recover its constitutive activity produced by the gain-of-function mutation. The *let-2(n821)* mutation undoubtedly alters the interaction between LET-2 and UNC-105, but it certainly does not disrupt the linkage between the two molecules as postulated by Liu *et al.* (1996) because, if so, the degradation of the collagen network by collagenase should have induced the same effect on channel activity as the *let-2(n821)* mutation. One possible interpretation of our findings is that (i) wild-type LET-2 is linked to mutant UNC-105(n506) without influencing its gating; (ii) the *let-2(n821)* mutation modifies the interaction between the two proteins resulting in the closure of the channel; and (iii) the disconnection of LET-2(n821) from UNC-105(n506) allows the channel to again freely open (Fig. 5). Finally, the experimental demonstration of an interaction between collagen and UNC-105 indicates that our open worm preparation preserves the functional integrity of the links between extracellular matrix and muscle cells. This is another convincing argument that the absence of a mechanosensory amiloride-sensitive response cannot be imputed to the possible disruption of mechanical links provided by the extracellular matrix network under our experimental conditions.

One possible interpretation of our results is that the connection between LET-2 and wild-type UNC-105, which we demonstrated to be electrically silent in resting muscle, may fulfil a structural function, probably redundant, since the *unc-105* null phenotype is wild-type (Park & Horvitz, 1986*a*). Collagen IV is a major component of basement membranes and staining for

LET-2 in *C. elegans* revealed its strong expression in the body wall muscle basement membrane lying between muscle cells and hypodermis (Graham *et al.* 1997). UNC-105 and LET-2 may thus participate in a transmembrane complex in charge of anchoring the muscle cells to the cuticle, relayed by hypodermal hemi-desmosome structures (Francis & Waterston, 1991). This may transmit the tension from muscles to the cuticle required for locomotion. From a phylogenetic point of view, UNC-105 is thought to be the most ancestral degenerin. It can be suggested that the ion channel function of the degenerin might have been lost in muscle while it became highly specialized in a more elaborated touch cell complex in neurones or stretch receptor complex in motor neurones.

This *in situ* electrophysiological study sheds new light on the degenerin properties and their physiological role in the native cellular environment. Our data probably suggest that the muscle degenerin UNC-105, although functionally interacting with a collagen, does not function as a stretch-regulated amiloride-sensitive Na^+ channel in *C. elegans* body wall muscle. Yet, muscle stretch activation is considered as one of the key mechanisms in the alternative ventro-dorsal sinusoidal propagating waves of muscle contraction, at least in the other nematode *Ascaris* (Turner, 2001). Our results suggest that other muscular stretch receptive structures might operate in *C. elegans* as indicated by our hypo-osmotic experiments, together with stretch receptive motor neurones which are also likely to assist this function (White *et al.* 1986; Tavernarakis *et al.* 1997). The elegant genetic dissection of the sensory mechanotransduction complex in *C. elegans* neurones suggests that the degenerin channels are the sensory transducers. Recent *in vivo* Ca^{2+} imaging experiments demonstrated that degenerins are involved in the Ca^{2+} transients evoked by gentle touch in neurones (Suzuki *et al.* 2003). The next challenge is to determine whether these degenerins do function as amiloride-sensitive stretch-regulated channels in *C. elegans* sensory neurones through the necessary parallel approaches of *in situ* electrophysiology as used in this work.

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Supplementary material

The online version of this paper can be found at:

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It consists of two movies. Movie no. 1 illustrates the changes in the shape of the muscle cell recorded in Fig. 2*B*, in response to the shifting of the glass rod used to flatten the cuticle of the worm. Movie no. 2 illustrates the changes in the shape of the muscle cell recorded in Fig. 2*C*, in response to the ejection of external solution from a glass capillary. The total duration of the movies no. 1 and 2 is 27 and 17 s, respectively. The timer is stamped in the bottom right corner.

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